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Molecular Cytogenetic Characterization of Early and Late Renal Cell Carcinomas in Von Hippel-Lindau Disease

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Deletions of 3p25, gains of chromosomes 7 and 10, and isochromosome 17q are known cytogenetic aberrations in sporadic renal cell carcinoma (RCC). In addition, a majority of RCCs have loss of heterozygosity (LOH) of the Von Hippel-Lindau (*VHL*) gene located at chromosome band 3p25. Patients who inherit a germline mutation of the *VHL* gene can develop multifocal RCCs and other solid tumors, including malignancies of the pancreas, adrenal medulla, and brain. VHL tumors follow the two-hit model of tumorigenesis, as LOH of *VHL*, a classic tumor suppressor gene, is the critical event in the development of the neoplastic phenotype. In an attempt to define the cytogenetic aberrations from early tumors to late RCC further, we applied spectral karyotyping (SKY) to 23 renal tumors harvested from 6 unrelated VHL patients undergoing surgery. Cysts and low-grade solid lesions were near-diploid and contained 1–2 reciprocal translocations, dicentric chromosomes, and/or isochromosomes. A variety of sole numerical aberrations included gains of chromosomes 1, 2, 4, 7, 10, 13, 21, and the X chromosome, although no tumors had sole numerical losses. Three patients shared a breakpoint at 2p21–22, and three others shared a dicentric chromosome 9 or an isochromosome 9q. In contrast to the near-diploidy of the low-grade lesions, a high-grade lesion and its nodal metastasis were markedly aneuploid, revealed loss of *VHL* by fluorescence in situ hybridization (FISH), and contained recurrent unbalanced translocations and losses of chromosome arms 2q, 3p, 4q, 9p, 14q, and 19p as demonstrated by comparative genomic hybridization (CGH). By combining SKY, CGH, and FISH of multiple tumors from the same VHL kidney, we have begun to identify chromosomal aberrations in the earliest stages of VHL-related renal cell tumors. Our current findings illustrate the cytogenetic heterogeneity of different VHL lesions from the same kidney, which supports the multiclonal origins of hereditary RCCs.

INTRODUCTION

Loss of heterozygosity at 3p25, gains of chromosomes 7 and 10, and an isochromosome 17q are among the few cytogenetic aberrations identified in early, low-stage sporadic clear cell renal cell carcinomas (RCCs) (Anglard et al., 1991; Dijkhuizen et al., 1997; Elfving et al., 1997; Wada et al., 1997). Advanced RCCs are characterized by hyperdiploidy or marked aneuploidy, with chromosomal aberrations involving all autosomes and with nonrandom changes most commonly mapped to 1p, 2q, 3p, 5q, 6q, and 8p (Mitelman, 1994; Presti et al., 1996; www.ncbi.nlm.mitelmansum.cgi). A molecular cytogenetic characterization of the same cancer at different stages may help delineate the pathways of tumorigenesis and eventually allow one to predict the clinical course from genotypic information.

Von Hippel-Lindau disease (VHL) has become an invaluable human model for dissecting the genetics of tumor initiation and progression. VHL is inherited as an autosomal dominant trait with variable penetrance and is characterized by vascular, cystic, and solid tumors of the central nervous system, retina, adrenal medulla, pancreas, wolffian duct structures, and the renal epithelium. All patients with VHL have a germline mutation, rearrangement, or deletion of the *VHL* locus at 3p25 (Zbar et al., 1996). In tumorous VHL kidneys, normal tubular structures can be seen juxtaposed to dysplastic tubules, small clear cell cysts, oligocellular cysts, small, low-grade solids, high-grade solids, or sarcomatoid tumors of high metastatic potential.

Among family members who share the same germline mutation, the expressivity of VHL can be surprisingly broad and can range from cystic kidneys to high-grade renal cell carcinomas with metastatic potential. Therefore, the phenotypic differences may be related to the accumulation of somatic mutations in tumors (Knudson, 1971; Zbar et al., 1987; Lubensky et al., 1996).

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TABLE 1. Patients Analyzed*

Patient	Sex	Age	Germline mutation	VHL sites
1	XX	54	Partial deletion, exons 1,2	CNS, kidney
2	XY	42	Missense, G→C, 553	CNS, kidney, epididymis
3	XY	62	Partial deletion, exon 3	CNS, kidney, pancreas, eye, epididymis
4	XX	27	Missense, A→G, 446	CNS, kidney, pancreas, adrenal
5	XX	38	Missense, C→T, 712	CNS, kidney, pancreas, adrenal
6	XY	39	Missense, T→C, 415	CNS, kidney, eye, metastases

*Missense mutations denoted with specific nucleotide change and partial deletions (rearrangements) indicated with exon deleted.

To identify chromosomal aberrations, we have applied spectral karyotyping (SKY) to metaphase cells from early (cysts and low-grade tumors) and late (high-grade and metastasic) VHL tumors. SKY allows the simultaneous visualization of all chromosomes in unique colors (Schröck et al., 1996; Veldman et al., 1997). In order to assess the consequence of chromosomal aberrations detected by SKY with respect to the acquisition of genomic DNA gains and losses in the tumors, we also analyzed appropriate cases by comparative genomic hybridization (CGH). CGH allows one to map regions of genomic imbalance to normal metaphase chromosomes (Du Manoir et al., 1997).

MATERIALS AND METHODS

Six unrelated VHL patients with known germline *VHL* mutations underwent surgery for uni- or bilateral renal lesions > 3 cm (Table 1). Partial nephrectomy in patients 1–5 and radical nephrectomy in patient 6 were performed (Fig. 1A). At operation, the primary lesion and all other gross or ultrasonographically identified solid tumors and cysts were removed as part of a strategy to extend the tumor-free period for each kidney (Walther et al., 1995). Within 2 hr after removal, tumors were archived, prepared for pathologic analysis, or minced and placed in renal epithelial growth medium (REGM; Clonetics/Bio-Whittaker, Walkersville, MD) in 5% CO_2 at 37°C. Patient 3 had two cell cultures (culture 1, passage 18; culture 2, passage 3) obtained from the same primary tumor after long-term storage in 10% DMSO and 10% fetal calf serum (FCS). In total, 23 cell cultures were successfully carried beyond passage 3 and processed for metaphase chromosomes as described (Veldman et al., 1997).

Metaphase chromosomes were hybridized with SKY kits prepared from flow-sorted chromosomes and detected 48 hr later as described (MacVille et al., 1997a). Image acquisition of metaphase cells was performed with SkyView software (Applied Spectral Imaging, Migdal Haemek, Israel). The spectral cube and a charge-coupled device camera (Hamamatsu, Bridgewater, NJ) were connected to a DMRXA microscope (Leica, Wetzlar, Germany) equipped with a custom-designed SKY-3 optical filter (Chroma Technology, Brattleboro, VT).

DNA was prepared from either cells in culture or primary formalin- or ethanol-fixed tumor lysates under salt-free conditions. For CGH, one microgram of biotin-labeled tumor DNA and digoxigenin-labeled, sex-matched normal donor lymphocyte DNA were cohybridized on sex-matched normal human lymphocyte metaphase chromosomes. Hybridization, detection, and image acquisition were performed as described with Q-CGH (Leica Imaging Systems, Cambridge, U.K.) or Cytovision (Applied Imaging, Newcastle upon Tyne, U.K.) software (Ghadimi et al., 1999). Ratios of tumor to reference signal of < 0.75 and > 1.25 were interpreted to represent a loss and gain, respectively, of DNA mapped to a particular chromosomal region. For a comprehensive description of the quantitative analysis of CGH, see Du Manoir et al. (1997).

Enumeration of *VHL* copy number by FISH was performed on metaphase cells obtained from tumors in culture that contained clonal aberrations as identified by SKY. cDNA probes were prepared from biotin-labeled cosmid clones 3, 11, or 31 as described (Kuzmin et al., 1994), cohybridized with chromosomal painting probes or centromere enumeration probes (CEP 2 or 7, Vysis, Downers Grove, IL) for a given aberration. A somatic loss of *VHL* was scored when a tumor had either > 15% of cells with monosomy 3 or had > 15% of cells with disomy 3 but only one *VHL* signal at 3p21 (Moch et al., 1998; Siebert et al., 1998). Digoxigenin-labeled phage DNA (P1-191), which hybridizes a > 15 kb DNA region, including and telomeric to *VHL*, was used as a positive control.

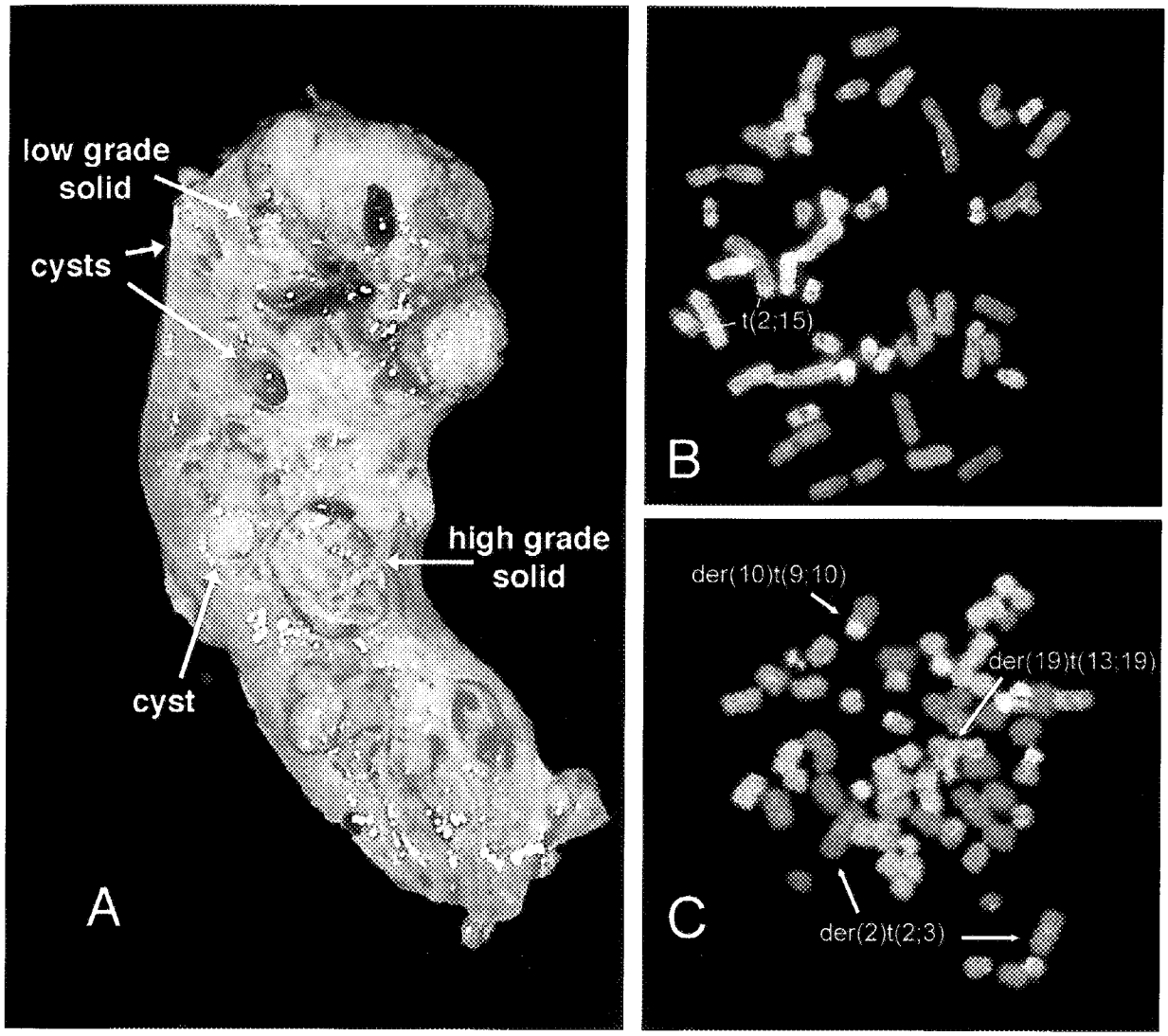

Figure 1. **A:** Sectioned nephrectomy specimen from VHL patient 6 illustrating some of the different tumor subtypes that exist concurrently in the VHL kidney. **B:** SKY of a low-grade VHL RCC from patient 1, lesion 2, reveals a near-diploid cell with a t(2;15)(p22;q22). By contrast, in **C**, SKY of a high-grade VHL RCC from patient 6, lesion 5, reveals marked aneuploidy and nonreciprocal translocations including der(10)t(9;10), der(19)t(13;19), and der(2)t(2;3) as denoted by arrows.

RESULTS

SKY of VHL Tumors in Culture

Eighteen of 23 cell cultures derived from the six patients listed in Table 1 contained a wide variety of nonclonal and clonal chromosomal aberrations. Five cell cultures from cysts and low-grade solid tumors revealed normal karyotypes (46 XX or XY, data not shown) or 5%–10% incomplete cells without aberrations. Fifteen low-grade tumors and cysts were near-diploid, had 1–2 chromosomal gains, and 1–2 reciprocal translocations, telomeric associations, or dicentric chromosomes (Tables 2 and 3). Recurrent numerical aberrations shared among different tumors of all grades included gains of the X chromosome and chromosomes 1, 2, 7, 10, 13, 21, and the X chromosome, and losses of 3, 8, 9, and 13–22. The few recurrent structural aberrations occurred in six tumors from four patients and included reciprocal and/or nonreciprocal translocations involving chromosomes 1, 2, 3, 9, 10, 13, and 15–19. In general, SKY revealed that cell cultures from low-grade lesions at passage 3–5 tended to yield low numbers of clonal aberrations. In contrast, low-grade lesions at seven or greater passages, or high-grade lesions at any passage, yielded recurrent aberrations in more than 30% of metaphase cells.

TABLE 2. Patients 1 to 3*

Patient 1
Lesions
1 Solid, low-grade, passage 9:44–46,XXder(18)t(1;18)(q30;q22)[2],cp[2]/46,XX[3]
2 Solid, low-grade, passage 9:43–46,XX **t(2;15)(p22;q22)[3]**,+7[2],+10[2],cp[5]
3 Cyst, passage 7:47,XX,+X[3]/46XX[2]
4 Cyst, passage 7:30–43,XX−2[3],−3[3],−7[3],−12[3],−13[3],−14[3],−16[3],−17[4],der(17)t(17;21)(p11;q?)[1],t(17;21)(p11;q22)[1],−20[3],−21[4],−22[3],cp[7]
5 Solid, low-grade, passage 7:32–45,XX,−9[3],+13[2],−18[4],cp[4]

Patient 2
Lesions
1 Cystic/solid, passage 3:41–47,XY,+7[5],−19[4],cp[5]
2 Cyst, passage 3:34–46,XY,+1[2],+2[2],−3[3],t(3;17)(p12;p11)[2],−5[4],−9[3],−10[3],−13[5],−14[4],−15[5],−18[3],−21[4],−22[3]cp[8]
3 Solid, low-grade, passage 3:37–46,XY,**t(2;X)(p22;?)[1]**,+7[8],−14[4],−19[4],−21[3],cp[9]

Patient 3
Lesions
1 Solid, low-grade, passage 18:44–47,XY+2[17],−7[3],−8[3],−13[4],−14[3],−15[3],−19[5],−20[6],−21[6],−22[5],cp[19]; passage 3:31–53,XY,**der(2)t(2;10)(p21;?p13)[1]**,−9[3],−15[3],−18[4],−19[5],−22[4],cp[5].

*Composite karyotypes by lesion number, histology, grade where appropriate, and cell culture passage number when analyzed. Clonal and/or nonclonal aberrations involving 2p21–22 in bold.

TABLE 3. Patients 4 to 6*

Patient 4
Lesions
1 Cyst, passage 3:42–47,XX,+1[3],+2[5],−4[4],−7[3],**dic(9;9(p10;p10)[1]**,+13[5],−14[3],−17[4],−19[4],−20[3],−21[5],cp[10]
2 Solid, low-grade passage 5:35–54,XX,−X[4],+1[2],t(3;X)(p21;q22)[1],der(4)t(4;15)(p14;q22)[1],−5[4],−7[3],−8[3],−9[3],+1[3],−12[9],−13[7],−14[6],−15[5],−16[9],t(16;19)(p?;p?[2],−17[5],−19[9],−20[5],−21[7],−22[6],cp[14]

Patient 5
Lesions
1 Solid, low-grade, passage 20:33–59,XX,dic(X;15)(q28;q26)del(X)(p11.4)[1],der(1)t(1;11)(p32;q23?)[1],dic(1;4)(q23;p15.3)del(1)(p32)[1],+2[2],+3[2],+4[2],tas(4;20)(p16;q13.3)[2],−6[8],−7[2],−9[8],der(9)t(3;9)(?;p12)[1];**i(9)(q10)[6]**,idic(9;17)9q22→9q10::9q10→9q22::17p11→17qter)[1],+10[2],−11[6],der(11;12)(p10;p10)[1],der(11)t(11;17)(p13;p12)del(11)q13)[1],der(11)t(11;18)(p11.2;p11.2)[1],−16[3],−17[4],dic(17;19)(p12;q13.3)[1],−18[9],der(18)t(16;18)(q11;q11)[1],der(18)t(17;18)(q12;q11.2)[1],tas(18;19)(q23;q13.4)[1],−19[5],−21[4],−22[3],cp[12]

Patient 6
Lesions
1 Cyst, passage 3:42–48,XY,+2[7],+17[2],cp[7]
2 Solid, low-grade, passage 5:36–40,XY,−Y[3],+2[2],−16[4],−17[5],cp[6]
3 Solid, low-grade, passage 9:26–42,XY,−1[5],t(1;2)(q11;p11)[5],−2[5],−5[3],−8[3],−11[3],−12[3],−13[3],−15[3],−16[3],−17[3],−18[4],−19[5],−20[5],−21[5],−22[5],cp[5]
4 Solid, high-grade, passage 3:35–47,XY,+2[4],−3[3],−21[3],−22[4],cp[8]
5 Solid, high-grade, passage 7:31–75,XY,+X[3],+Y[4],+1[2],−2[4],**der(2)t(2;3)(q31;**q12)[9],−3[5],+5[5],+6[8],+7[5],+8[3],−8[4],i(8)(q10)[4],−9[7],der(10)t(9;10)(q31;q33)[9],idic(9)(q13)[2],+10[2],+11[5],+12[7],−13[3],−14[8],+15[3],+16[4],−17[4],−18[6],+19[3],−19[3],der(19)t(13;**19)(q31;p13.1)[2]**,+20[5],+21[5],der(22)t(4;1;22;1)(p14;p34;p11.2;p24)del(1)p21-31,[2],−22[7],cp[10].
6 Metastatic lymph node, passage 7:45–72,XY,+X[3],+1[4],der(2)t(2;3)(q31;q12)[4],+5[3],+6[2],+7[4],+8[6],i(8)(q10)[3],**der(10)t(9;10)(q31;q33)[3]**,+10[4],+11[5],+12[6],+15[4].+16[6],+17[2],+18[2],der(19)t(13;19)(q31;p13.1)[6],+20[4],+21[5],+22[4],cp[7]

*Composite karyotypes of all aberrations seen. Clonal and/or nonclonal aberrations leading to 9p loss in bold.

SKY detected a translocation involving chromosome band 2p21–22, which occurred in patients 1, 2, and 3 (Table 2). Patient 1 had a low-grade tumor cell culture (passage 3), with 3 of 10 metaphase cells (30%) having a t(2;15)(p22;q22) without other aberrations (Fig. 1B). Two other low-grade solid tumors from the same kidney were +X and +13. Patient 2 had a t(X;2)(p22;p22) in one of 10 (10%) metaphase cells from a passage 3 cell culture derived from a low-grade solid tumor. Eight of the nine remaining cells showed only trisomy 7. A second low-grade tumor from the kidney had a t(3;17)(p12;p12) in one of eight cells. A tumor from patient 3 had +2 in 17 of 19 (88%) cells (passage

18), and CGH revealed a gain of chromosome 2 as the only imbalance. A second culture from the same tumor at passage 3 revealed der(2)t(2;10)(p21;q?13) in 1 of 10 cells.

A variety of aberrations involving 9p were seen in patients 4, 5, and 6 (Table 3). Patient 4 had a cell culture from a clear cell cyst with a dic(9;9)(p10;p10) in 10% of metaphase cells, as well as +1 in 30%, and +2, +13, and −21 in 50%.

Patient 5 had a low-grade solid lesion at passage 10, which revealed i(9q)(q10) in 6 of 12 (50%) cells. Each of the 12 cells evaluated was different karyotypically. However, chromosomes 4, 9, 11, and 18 were involved in 11 of 13 identified aberrations, including a variety of dicentric, isocentric, and isodicentric chromosomes, telomeric associations, and reciprocal translocations.

Patient 6 underwent a radical nephrectomy, which revealed a severely diseased kidney with multiple small cysts, low- and high-grade solid tumors, and metastatic regional lymph nodes (Fig. 1A). The patient succumbed to metastatic disease 6 months postoperatively. At surgery, 34 lesions were harvested and cell cultures were established. Metaphase cells could be prepared successfully from a total of six cell cultures at passages 3–7. Lesion 1 (a cyst) and lesions 2 and 4 (low- and high-grade solid tumors, respectively) revealed trisomies of chromosome 2 in 30%–100% of metaphase cells, with variable losses of other autosomes. As shown in Figure 2A, CGH revealed sole gains of chromosome 2 in lesion 2. Lesion 3 was a low-grade solid tumor with a reciprocal translocation involving 1q11 and 2p11. None of the cells had trisomy 2. CGH revealed gain of chromosome 1 from 1qter→1p20 and gain of chromosome 2 from 2qter→2p20 (Fig. 2B). Lesion 5 was a high-grade lesion that had spread widely throughout the kidney parenchyma and contained four recurrent, nonreciprocal translocations: der(2)t(2;3)(q21;q11), der(10)t(9;10)(q31;q33), der(19)t(13;19)(q31;q13.3), and idic(9)(q13) (Fig. 1C). CGH revealed gains of all autosomes, except 16, 21, and 22, and losses of 2q31→2qter, 3q11→3pter, 9q11→9pter, and 10q18→10pter (Fig. 2C). Lesion 6 was a perirenal metastatic lymph node, which had the same der(2)t(2;3) and der(10)t(9;10) as the primary tumor (lesion 5). Although two other nonreciprocal translocations were identified—der(16)t(16;22)(p10;q11) and der(4)t(4;13)(q21;q11)—the CGH profile of the lymph node culture had no significant differences from that of lesion 5. In comparison, CGH of a formalin-fixed liver metastasis obtained at autopsy from the same patient revealed the same 2q and 3p losses seen in the cell lines of the primary tumor, as well as gains of the X chromosome and chromosome 5 and losses of chromosomes 9 and 10 (data not shown).

FISH Analysis of VHL

FISH using *VHL*-specific probes and chromosome-specific painting probes was applied to metaphase cells to assess for *VHL* locus copy number. We restricted analysis to those metaphase cells which had a clonal structural or numerical aberration as identified by SKY. For example, in patient 6, lesion 3, a clonal t(1;2) was seen by SKY. We used a chromosome 2 painting probe to identify on slides from the same tumor preparation those metaphases that carried t(1;2), or corresponding derivative chromosomes. Signal number for *VHL* on chromosome 3 was enumerated on these aberrant metaphases only. With this strategy applied to other tumors, loss of *VHL* was seen in 10% of cells with +7 in lesion 1, patient 2. Loss of *VHL* was seen in 0, 0, and 11% of cells with +2 in lesion 1, patient 3, or lesions 1 and 4, patient 6 (Table 4), respectively. In lesion 3, patient 6, nine metaphase cells with two chromosomes 3 and also t(1;2)(q11;p11) were identified, and loss of one signal for *VHL* was seen in one (Table 4, Fig. 3). In lesions 5 and 6, patient 6, a total of 10 and 6 cells were identified with der(2)t(2;3)(q31;q12), respectively. Of these cells, approximately 65% were hypertriploid and had two copies of chromosome 3 and two signals for *VHL*; 25% had monosomy 3 (with a signal for *VHL*), and 10% were incomplete and/or hypodiploid,

DISCUSSION

Spectral karyotyping was used to facilitate the search for cytogenetic changes during renal cell carcinogenesis from patients with VHL. SKY has been performed in our laboratory on cell lines of pancreatic and colorectal tumors (Ghadimi et al., 1999, 2000), breast and cervical cancers (MacVille et al., 1997b; Ried et al., 1997), and transitional cell carcinomas of the bladder (Padilla-Nash et al., 1999). The results revealed markedly aneuploid lesions with high numbers of nonreciprocal translocations and numerical aberrations that resulted in tumor-specific DNA gains and losses. In contrast to these studies of established cell lines, we applied SKY to primary cell cultures of 18 renal cell carcinomas and cysts from six patients with VHL to compare the cytogenetic changes from low- (cysts; small, solid tumors) to high-stage lesions (large, solid tumors; metastases). However, even a very careful dissection of the uni- or oligocellular clear

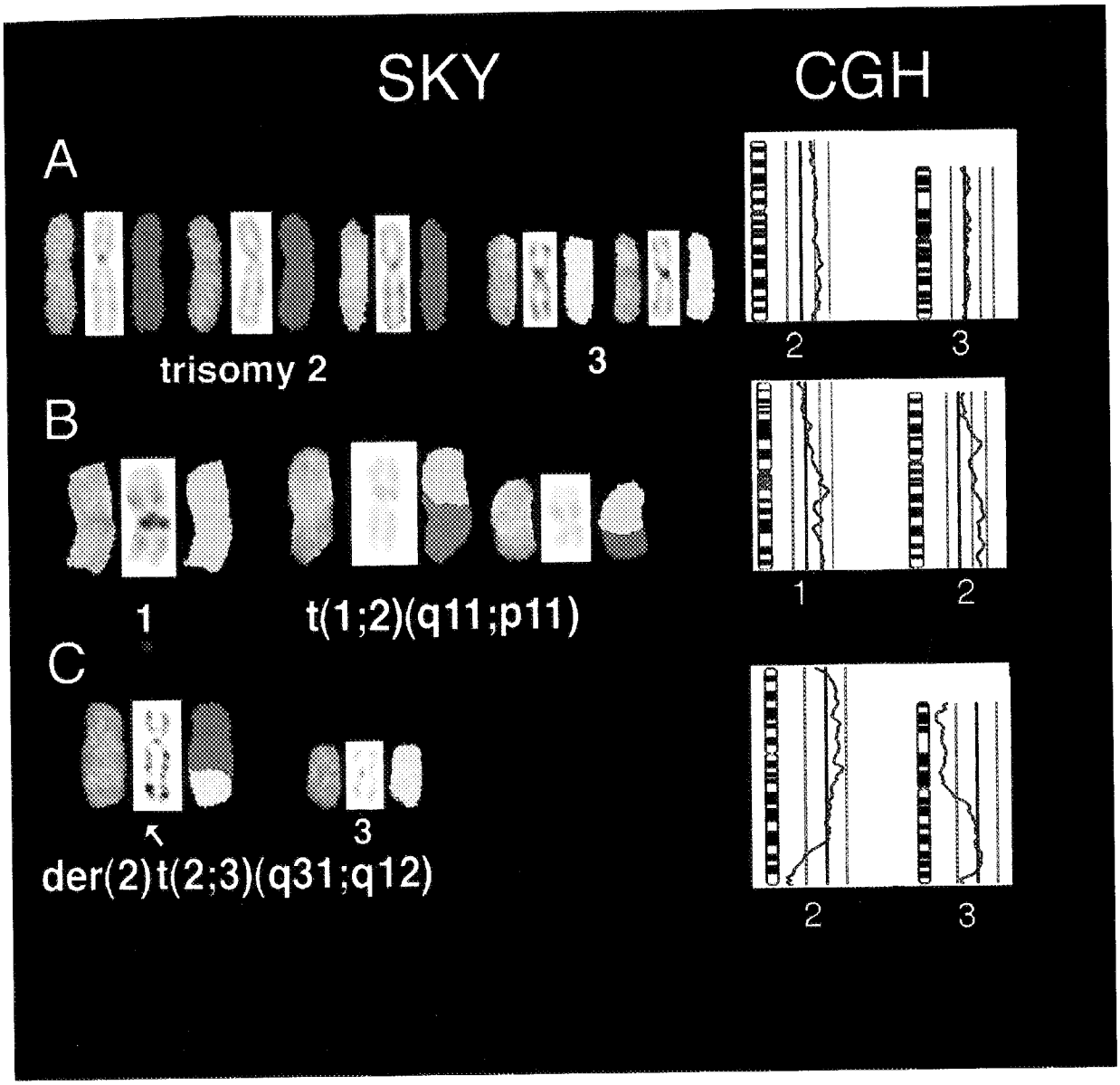

Figure 2. Aberrations of chromosome 2 in three different tumors from the same kidney of VHL patient 6. **A:** Lesion 2, a low-grade, near-diploid solid tumor with trisomy 2. CGH shows a gain in chromosome 2. **B:** Lesion 3, a low-grade, near-diploid solid tumor with t(1;2)(p11;q11.2). CGH shows gain of 1p20→1qter and 2p20→2qter. **C:** Lesion 5, a high-grade, markedly aneuploid solid tumor with multiple translocations including der(2)t(2;3)(q21;q21). CGH illustrates a marked 3p loss, the hallmark of classic sporadic clear cell renal cell carcinoma, seen in this and other high-grade lesions of patient 6.

cell linings from VHL cysts for culture may not preclude contamination by normal cells (Linehan et al., 1989), a small percentage of which may contain inconsequential chromosomal gains (Casalone et al., 1992; Elfving et al., 1995; Knuutila et al., 1995). In addition, it is unknown if nontumorigenic but *VHL*-haploinsufficient cells may develop karyotypic abnormalities in vivo or in vitro. With these caveats in mind, we interpret our SKY data, which revealed a copious cytogenetic heterogeneity between tumors from the same diseased kidney, as further evidence of the multiclonal nature of hereditary renal cell carcinoma.

Almost all of our low-grade or low-stage (early) lesions could be characterized by the presence of few reciprocal translocations, the presence of translocations involving 9p, or by a gain of chromosome 2. In contrast, the pattern of cytogenetic changes and the high degree of aneuploidy in high-grade VHL tumors resembled the pattern seen in sporadic renal cell carcinomas, which included multiple, unbalanced translocations and DNA gains and

losses involving chromosomes and chromosome arms 1p, 2q, 3p, 4, 7, 9p, 10, 13, 17p, and 22.

Trisomy 2 was seen in seven tumors from three patients; in two cysts and a low-grade solid tumor, trisomy 2 was the sole recurrent chromosomal gain. Trisomy 2 has been previously described in late renal cell carcinomas, although not as a sole abnormality and among a varying degree of aneuploidy (Mitelman et al., 1994). We are pursuing the identification of trisomy 2 in uncultured cells with FISH using centromere enumeration probes (CEPs) on tissue imprints of tumors that had trisomy 2 in > 30% of metaphase cells by SKY. Our preliminary data suggest that trisomy 2 can be found in a low number (< 5%) of uncultured cells derived from histologically defined clear cell carcinomas from VHL lesions. This suggests that trisomy 2 may convey an in vitro selection advantage for a previously uncharacterized but low-frequency population of preneoplastic or neoplastic cells in the VHL kidney. However, we cannot exclude that trisomy 2, as some have shown with chromosomes 7 and 10, is an in vitro phenomenon unrelated to tumorigenesis (Casalone et al., 1992; Elfving et al., 1995; Knuutila et al., 1995).

TABLE 4. *VHL* Copy Number by FISH Performed on Metaphase Cells From Tumors that Had Clonal Aberrations (Markers) by SKY*

Patient	Tumor	Marker	n	Loss *VHL*	% loss
2	1	+7	18	2	11
3	1	+2	9	0	0
5	1	4, 11[a]	12	1	<8
6	1	+2	12	0	0
6	3	t(1;2)	9	1	11
6	4	+2	14	1	7
6	5	der(2)t(2;3)	10	3	30
6	6	der(2)t(2;3)	6	4	67

*Enumeration stategy described in text.
[a]Markers were tas(4;20) or der(11).

Patient 6 in our series had tumors harvested from one kidney representing the spectrum of VHL disease from cystic lesions to metastases. A cyst and two low-grade lesions revealed gains of chromosome 2 as discussed above, while another low-grade tumor with a reciprocal t(1;2)(p11;q11.2) had

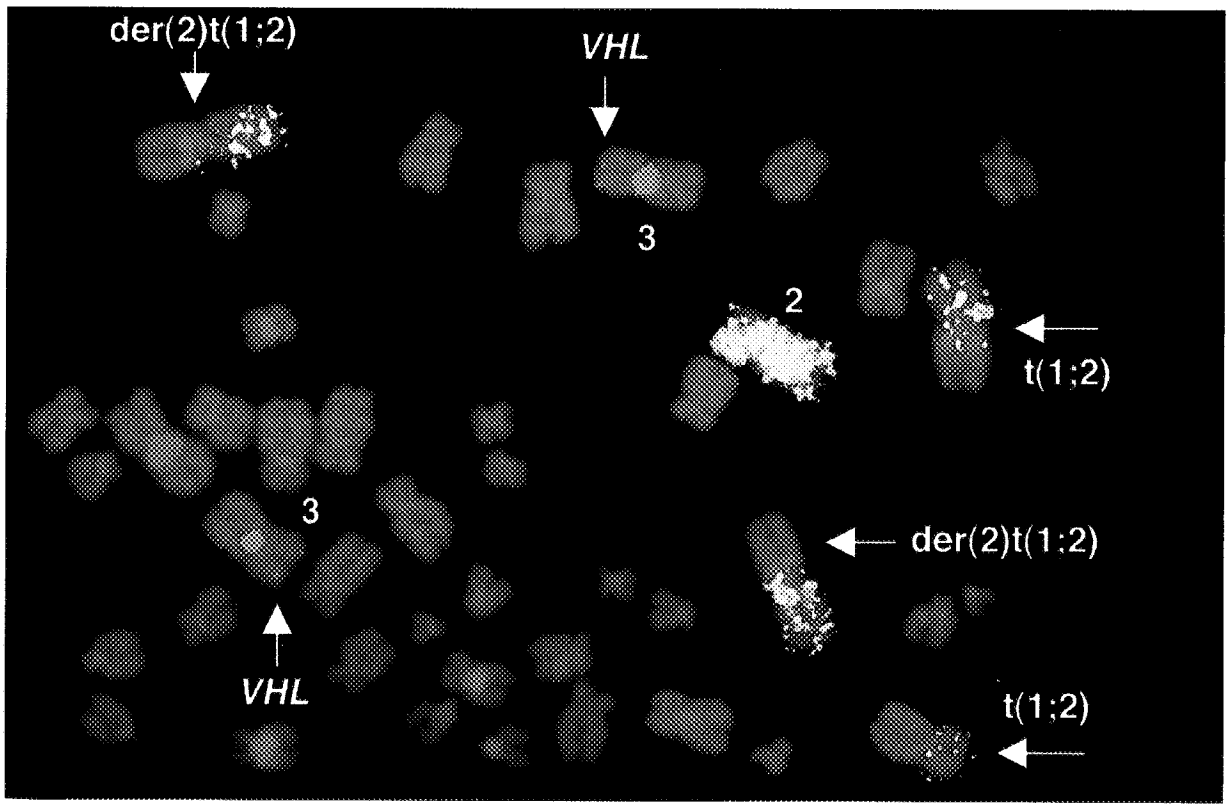

Figure 3. *VHL* copy number by FISH in cultures with clonal aberrations. Shown is lesion 3, patient 6, hybridized with cosmid 11 *VHL* probe (blue), centromere enumeration probe (CEP) for chromosome 3 (green), and chromosome 2 painting probe (yellow). FISH reveals disomy 3, t(1;2)(q11;p11) revealed by SKY and two signals for *VHL* (Table 4). This cell also has der(2)t(1;2) × 2.

net gains of 1p11→1qter and 2p11→2qter. In contrast, a high-grade tumor had a nonreciprocal der(2)t(2;3)(q31;q12) and resulted in the loss of 2q21→2qter and a whole arm loss that mapped to 3p. Deletions and LOH of loci on chromosome arms 2q and 3p have been demonstrated in carcinomas of the lung, colorectum, and sporadic RCC (Takita et al., 1995; Otsuka et al., 1996; Presti et al., 1996). Whether *VHL* is a target in these tumors remains to be established.

In VHL and sporadic renal cell carcinoma, 3p deletions, such as the whole arm loss demonstrated in patient 6, lesions 5, 6, and liver metastasis are the proposed mechanism of somatic inactivation of *VHL*, the second hit of the two-hit hypothesis in the initiation of renal neoplastic development (Knudson, 1971; Zbar et al., 1987, 1996; Lubensky et al, 1996). Our FISH data failed to demonstrate a consistent loss of *VHL* in low-grade lesions with clonal aberrations such as trisomy 2, trisomy 7, or t(1;2), but revealed monosomy 3 in a high-grade lesion and its metastasis. Methylation of *VHL* has been established as a mechanism of somatic inactivation in 7% and up to 30% of hereditary and sporadic RCCs, respectively, and may play a role in the cystic and low-grade tumors of VHL disease (Prowse et al., 1997; Clifford et al., 1998). An alternative hypothesis to be tested suggests that tumors in VHL disease first develop small deletions of 3p, which become larger as the tumor becomes more aneuploid (Prowse et al., 1997). Somatic *VHL* deletions in early (low-grade) lesions may be better assessed, therefore, with *VHL* allelotyping; therefore, we are currently allelotyping DNA from cultured and uncultured, whole and microdissected VHL cysts and low- and high-grade solid VHL tumors.

The precise sequence of cytogenetic events during the genesis of VHL-related RCCs and the relevance of sporadic numerical chromosomal aberrations in early lesions remain to be determined. SKY has been invaluable in demonstrating subtle and rare chromosomal aberrations in primary cell cultures, a particularly demanding sample type using classic techniques. Histologically defined low-grade lesions had few clonal aberrations, consistent with the multifocal origin of VHL-associated tumors. Persistent genetic instability, however, promotes the acquisition of additional clonal chromosomal aberrations to the extent that advanced-stage VHL tumors ultimately resemble the cytogenetic aneuploidy of sporadic renal cell carcinomas.

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